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Hematological, biochemical and histopathological assessment of the toxicity potential of seed kernel extracts of *Mangifera indica* Linn. varieties in mice

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ABSTRACT

The aim of the research was to assess the toxicity potential of seed kernels extracts of *Mangifera indica* Linn varieties against the hematology, biochemistry, and histopathology in Swiss albino mice. The acute and sub-acute toxicity of *Opioro* and *Julie* varieties were assessed in Swiss albino mice, *in vivo*. In acute toxicity study, the Swiss albino mice were administered orally with each extract at single doses of 500, 1000, 2000, 3000, 4000 and 5000 mg/kg for 14 days while single doses of 400 mg/kg (low dose) and 800 mg/kg (high dose) of each extract of the varieties was given to the mice during sub-acute toxicity for 28 days. Blood sample was analyzed for hematological indices while blood serum was assayed for antioxidant and biochemical parameters. Kidney and liver were collected and assessed for histopathology. The lethal dose (LD_{50}) of *Opioro (3700 mg/kg)* and *Julie (4300 mg/kg)* extracts fell within the safety range. The biochemical, antioxidant and hematological parameters showed various levels of significance ($P<0.05$–$P<0.0001$). Evaluation of the organ histology in the Swiss albino mice, indicated that liver and kidney were unaffected by the extracts. This study demonstrated that use of the extracts could not produce serious toxic effect in the mice especially in low doses.

Key words: Mango, Mice, Acute toxicity, Sub-acute toxicity, biochemical, seed kernels.

1. INTRODUCTION

Nature, since antiquity has provided us with plants and plant-derived products that are necessary in enhancing the nutritional and health standards of humans. Most bioactive compounds used in food and drug-producing industries are the derivatives of plants (Anand *et al*., 2019). These plants are rich in essential nutrients (Radha et al., 2021). Due to the detrimental health effects of most chemical-dependent processed drugs, natural products have been advocated for, as excellent alternative due to new active ingredients they possess and required

for new drug development and treatment of infections (Newman and Cragg, 2020).

In fact, before the inception of contemporary medicine, the use of herbal plants and their products for treatment and remedial purposes have been in existence (Yuan *et al*., 2016). Their oils are rich in antioxidants, phytocompounds and other bioactive constituents which are considered safe for consumption; they are known also to have antimicrobial effect (Radha *et al*., 2021). Instances where chronic diseases exist, plant derived antioxidants intake has been effective in their management (Torres-Leon et al., 2016). Arogba and Omede, (2012) reported that the consumption of plant-derived antioxidants is innocuous in protecting the body against lifelong diseases resulting from oxidative reactions in the body.

Mangifera indica L. (Mango) is a perennial fruit plant that belongs to the family *Anacardiaceae* cultivated mostly in Asia, America and part of Africa. Currently, India is the largest producer of mango (Mwaurah *et al*., 2020). It is grown for its fruit, stem bark, leaves and peels. In developing countries, the leaves and stem bark have been used in preparing concoctions for bacterial infections, malaria and ulcerative inflammation (Awad El-Gied *et al*., 2012). Despite reports on the antibacterial properties of peels and pulp of mangoes, treatment with seed kernels of the same varieties is much better due to their elevated antioxidant and polyphenolic contents (Lebaka *et al*., 2021).

Seed kernels of *M. indica* L. serve as sources of food nutrients and anticancer agents for humans and feeds for livestock (Torres-Leon *et al*., 2016). Often, their milled samples are known to be curative agents for diabetes, hypertension, inflammation, diarrhea in animal models (Torres-Leon *et al*., 2016) and neutralization of venoms during snake bite (Abdel-Aty *et al*., 2018). The crude extracts could be applied topically in form of cream on the wound surface or taking orally as medications to eliminate pathogenic bacteria (Mutua *et al*., 2016; Anyanwu and Okoye, 2017). This approach however, has reduced antibiotic resistance among patients while improving their immune function.

Opioro and *Julie* mangoes are often consumed for their therapeutic and nutritional benefits. It is believed that both varieties share similar origin based on their dendogram report (Aguoru *et al*., 2016). However, despite the therapeutic potential, their toxicity profile to the best of our knowledge, has not been evaluated especially in liver and kidney. Therefore, there is a need to thoroughly research on the kernels to ascertain their safety levels.

2. MATERIALS AND METHODS

2.1. Sample collection and preparation of extracts

Three hundred (300) fresh fruits of *Opioro* and *Julie* varieties used for the study were obtained from Umuagbaghi village in Aba South LGA of Abia state. The fruits were taken to the Department of Microbiology Laboratory, Abia State University, Uturu. The taxonomist, Mrs. Chikodiri from the Department of Plant Sciences and Biotechnology, Abia State University, Uturu identified the samples with their leaves and fruits. Voucher specimens of *Opioro* (ABSU/PSB/250) and *Julie* (ABSU/PSB/251) varieties were deposited at the herbarium of the department. The juicy mesocarps of the two varieties were separated from the seed kernels manually with sharp knife. The seeds were removed from the kernels (endocarps) and were chopped, air-dried under shade at room temperature for 4 weeks and milled into powder using Panasonic electric blender (MX-AC210 model, 170 mm x 236 mm x 268 mm Panasonic, Japan). The milled samples were stored at 4^{o}C closed container and kept in a refrigerator.

2.2. Experimental animals

Adult male Swiss albino mice aged 4–6 weeks weighing 22–30 g used for the experiment obtained from the Department of Biochemistry animal house, Michael Okpara University of Agriculture, Umudike, Nigeria. The experiments were conducted according to internationally accepted principle on the use and care of laboratory animals (National Research Council, 2011). The mice were properly caged in a conducive and neat steel cages and fed with mice pelleted feed (vita finisher); clean water was given to them orally with gavage syringe. They were maintained under standard laboratory conditions of regular 12 h light/12 h dark cycle and temperature of 24±1 ^{o}C throughout the period of the experiment. The methods of the experiment were carried out in accordance with the National Research Council guideline for the care and use of laboratory animals (National Research Council, 2011).

2.3. Acute toxicity study

The acute toxicity study was performed using the method described by the Organization for Economic Cooperation and Development (OECD) 423 guideline (2001) with modification. For each extract, thirty male mice were used. Briefly, for *Julie*, the 30 male mice were randomly assigned to groups of 5 mice each. Each treatment group was administered with a specific dose level of the extract via oral route with gavage syringe according to the order: Group 1 (500 mg/kg body weight), group 2 (1000 mg/kg body

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weight), group 3 (2000 mg/kg body weight), group 4 (3000 mg/kg body weight), group 5 (5000 mg/kg body weight) and the control group (group 6). The control group was treated with the same volume of distilled water. After treatment, the mice were returned to their cages and allowed access to food and water but were observed for toxicity signs such as salivation, tremors, convulsion, breathing rate and mortalities within 24 h and thereafter once daily for the remaining 13 days. At the end of the period, the dose (LD_{50}) that caused 50% death of the male mice population for each group was determined.

2.4. Sub-acute toxicity study

The sub-acute toxicity study was performed on male mice as per OECD guideline 407 guideline (2001). The mice were also randomly divided into one control group and two experimental groups with 10 mice in each group. Group 1 (control group) received water orally whereas Groups 2 and 3 were fed with Julie extract doses of 400 mg/kg (low dose) and 800 mg/kg (high dose) respectively for 28 days. The same procedure was performed with *Opioro* extract for the same duration. The groups fed with *Julie* extract were kept separate from the groups fed with *Opioro* extract. After the 28th day, the experimental animals were starved overnight; under anesthesia, blood samples were collected through cardiac puncture for biochemical and hematological analysis. After euthanasia, the mice were sacrificed and the liver and kidneys of these animals were dissected for histopathological analysis.

2.5. Hematological analysis

The hematological analysis was performed using an automated hematological analyzer (Sysmex XS-1000i). Briefly, 1 mL of blood sample was collected through cardiac puncture with two sterile tubes containing ethylenediamine-tetracetic acid (EDTA) and one empty tube. The blood in the empty tube was centrifuged at 3000 rpm at 4°C for 10 min to obtain the serum for biochemical analysis, while hematological test was conducted with the blood in the EDTA bottle. The parameters analyzed were red blood cells (RBC), hematocrit (HCT), hemoglobin (Hb), white blood cell count (WBC), platelets, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) (Akomas *et al*., 2014).

2.6. Biochemical analysis

Liver function tests, renal function tests and lipid profile were performed with automated biochemical analyzer (Cobas Integra® 400 plus). The clinical biochemistry parameters analyzed were, total proteins (TP), albumin (ALB), globulin, alanine amino-transferase (ALT), aspartate amino-transferase (AST), alkaline phosphatase (ALP), biluribin, urea, creatinine. Total cholesterol, triglycerides (TAG), high density lipoprotein (HDL) were all performed while very low-density lipoprotein-cholesterol (VLDL-c) were calculated by the formula: TAG/5; low density lipoprotein-cholesterol (LDL-c) was determined by the formula: Total cholesterol-HDL-c + VLDL-c (Akomas *et al*., 2014).

2.7. Antioxidant enzyme assay

2.7.1. Determination of catalase activity

The catalase assay was determined using the procedure illustrated in (Aebi, 1984) with slight modification by Gorny *et al*. (2020). Briefly, 50 µL homogenate of liver was added to 650 µL of 50 mM phosphate buffer (pH 7.0), later, 300 µL of 54 mM H_2O_2 was added to the solution. After 3 min, the absorbance was measured at 240 nm. The activity of catalase in the liver was expressed in µmol/mg of protein. The solution used for control contains 50 mM phosphate buffer, pH 7.0 and 300 µL of 54 mM H_2O_2.

2.7.2. Determination of glutathione peroxidase (GPx) activity.

The activity of GPx was assayed with the Cayman's Glutathione Peroxidase Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA). The oxidized glutathione (GSSG) formation reaction catalyzed by GPx was coupled with the glutathione disulfide reductase (GR) reaction, which regenerates GSH at the expense of nicotinamide adenine dinucleotide phosphate (NADPH) oxidation. GPx activity was expressed in µg /mg of protein (Gorny *et al*., 2020).

2.7.3. Determination of superoxide dismutase (SOD) activity.

SOD activity was measured by colorimetric assay using Cayman's kit (Cayman Chemical Company, Ann Arbor, MI, USA). The working principle of this kit is the formation of superoxide by the xanthine/xanthine oxidase reaction and then the superoxide generated becomes reduced by SOD enzyme. SOD activity was expressed in µg /mg of protein (Gorny *et al*., 2020).

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2.7.4. Determination of malondialdehyde (MDA) activity.

The determination of MDA was according to the method of Ohkawa *et al*. (1979), with modification by Gorny *et al*. (2020). Two hundred and fifty µL of both 0.37% thiobarbituric acid (TBA) and 15% TCA were added to 125 µL of liver homogenate of Swiss Albino mice. The samples were stirred using vortex and incubated at 100 ◦C for 10 min. MDA in the homogenate was analyzed using a standard curve prepared with 25 µM 1,1,3,3-Tetraethoxypropane (TEP) and was expressed in µg /mg of protein per gram tissue.

2.7.5. Determination of reduced glutathione (GSH)

The activity of GSH was determined using the Cayman's Glutathione Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA). GSH activity was expressed as µg /mg of protein (Gorny *et al*., 2020).

2.8. Histopathology

Slices of the organs (liver and kidney) administered with the extracts of *Opioro* and *Julie* were fixed in 10% formal saline for 48 h. The specimens were processed by placing them in ascending graded series of alcohol (50%-100%) at interval of 1 h each to ensure proper dehydration of the tissues. The dehydrated tissues were then transferred to mixture of equal volumes of alcohol and xylene where they were left overnight and later cleared with two changes of xylene for 1 h each. They were then infiltrated twice for 1 hour each with molten paraffin wax in the oven at 60^0C. The tissues were then embedded in paraffin wax, trimmed and mounted on wooden chuck, and then taken to the microtome for sectioning at 5µm thickness. The sections were floated in floating-out bath from where they were picked with clean albuminized slides. The slides were placed in a staining dish and excess wax was removed by two changes of xylene, hydrated by descending graded series of alcohol (100%-70%) for 2 min each. The slides were taken to water and then stained by infiltrated Ehrlich hematoxylin for 15 min, and then washed in water for 5 min, differentiated in 10% acid alcohol and blued in running tap for 10 min. They were then counter stained with filtered eosin for 2 min. Excess eosin was removed in ascending graded series of alcohol (75%-100%) for 2 min each, cleared in two changes of xylene and each was covered with depex mountant. The slides were viewed under a light microscope and selected images were captured using moticam 2.0 digital camera attached to a computer (Akomas *et al*., 2014).

2.9. Statistical analysis

The results were expressed as means± SD using graph pad prism graphical statistical package version 5. The student t-test at $p<0.05$ was applied to assess the difference between the mean for two variables and two-way analysis of variance (ANOVA) for more than two variables followed by Bonferreni post hoc test.

3. RESULTS

3.1. cage-side study

In the acute toxicity study, the lower doses of 500 mg/kg, 1000 mg/kg and 2000 mg/kg of the two extracts did not show signs of toxicity or cause the death of any mice. However, at higher doses before the death of mice, they showed signs such as tremor, weakness, respiratory failure and convulsion. At 3000 mg/kg and 5000 mg/kg doses, *Opioro* extract produced 20% and 60% mortality while *Julie* produced 20% mortality at 5000 mg/kg.

3.2. Effects of the extracts on hematology parameters

All tested doses of *Opioro* and *Julie* extracts (400 and 800 mg/kg) induced a significant ($P<0.001$; $P<0.0001$) reduction in platelets compared to the control group except 400 mg/kg of *Julie* extract. At 400 mg/kg dose of *Julie*, the platelets as well as other parameters were not affected. There was no significant difference ($P>0.05$) observed in RBC, HCT, Hb, WBC, MCV, MCH and MCHC of groups of mice treated with *Opioro* and *Julie* extracts when compared to the control. Result of effect of sub-acute oral administration of *Opioro* and *Julie* extracts on hematological parameters in Swiss albino mice was presented in Table 1.

3.5. Effects of the extracts on liver function

ALT and AST were significantly high ($P<0.01$ and $P<0.001$) in mice administered with 400 mg/kg and 800 mg/kg doses of *Opioro* extract. Treatment with 400 mg/kg and 800 mg/kg of *Julie* extract resulted in a significant rise ($P<0.01$ and $P<0.001$) of ALT and AST when compared to the control. However, at 400 mg/kg of *Opioro* extract, there was a significant reduction ($P<0.001$) in ALP activity.

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There were no significant variations in the activities of total proteins, albumin, globulin and bilirubin when treated with both 400 and 800 mg/kg doses of the two extracts (Table 2).

Table 1

Effect of sub-acute administration of *Opioro* and *Julie* extracts of *M. indica* L. seed kernel on hematological parameters in male Swiss albino mice.

Parameters	**Control**	***Opioro***		***Julie***	
		400 mg/kg	**800 mg/kg**	**400 mg/kg**	**800 mg/kg**
RBC (10^6/mm^3)	5.41±0.18	5.05±0.22	4.88±0.16	5.23±0.15	5.04±0.07
HCT (%)	39.80±1.48	37.80±1.79	36.20±2.59	38.20±1.30	37.00±2.35
Hb (g/dL)	12.23±0.42	11.85±2.23	11.90±0.18	11.90±0.24	11.89±0.41
WBC (10^3/mm^3)	9.17±0.23	9.43±0.31	9.62±0.60	9.62±0.39	9.52±0.32
Platelets (10^3/mm^3)	93.68±10.86	82.72±2.57****	85.34±4.53***	90.20±6.06	85.48±3.85***
MCV (fL)	73.56±1.23	74.88±3.46	74.19±3.70	73.10±1.66	73.44±3.94
MCH (pg)	22.63±0.86	23.45±0.68	24.41±0.44	22.80±0.93	23.60±0.74
MCHC (g/dL)	30.75±1.29	31.40±1.58	32.97±1.91	31.19±1.14	32.22±2.01

Data were expressed as mean± SD, n = 5. Statistical analysis was performed using two-way ANOVA followed by Bonferreni post hoc test.

*** (P<0.001) compares with respective positive controls. **** (P<0.0001) compares with respective positive controls

RBC, red blood cells; HCT, hematocrit; Hb, hemoglobin; WBC, white blood cell count; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

Table 2

Effects of sub-acute administration of *Opioro* and *Julie* extracts of *M. indica* seed L. kernel on liver function indices in male Swiss albino mice.

Parameters	**Control**	***Opioro***		***Julie***	
		400 mg/kg	**800 mg/kg**	**400 mg/kg**	**800 mg/kg**
Total protein(g/dL)	5.82±0.14	5.66±0.16	5.79±0.38	5.62±0.45	5.68±0.17
Albumin(mg/dL)	3.56±0.24	3.02±0.16	3.032±0.08	3.52±0.12	3.20±0.17
Globulin(mg/dL)	2.262±0.24	2.64±0.25	2.76±0.34	2.09±0.52	2.48±0.11
ALT (IU/L)	24.80±2.95	27.00±1.58****	35.20±2.17**	27.20±1.30****	34.20±1.64**
AST (IU/L)	32.40±1.82	36.40±1.52**	41.20±1.92****	33.80±1.64	43.00±2.55****
ALP (IU/L)	71.00±2.92	65.80±2.68****	67.20±4.49	70.40±3.59	71.80±2.49
Bilirubin (mg/dL)	0.47±0.03	0.48±0.06	0.56±0.03	0.49±0.04	0.55±0.04

Data were expressed as mean± SD, n = 5. Statistical analysis was performed using two-way ANOVA followed by Bonferreni post hoc test.

** (P<0.01) compares with respective positive controls. **** (P<0.0001) compares with respective controls was done with two-way ANOVA followed by Bonferreni post hoc test.

ALT, alanine-amino transferase; AST, aspartate-amino transferase; ALP, alkaline phosphatase.

3.5. Effects of the extracts on lipid Profile

All tested doses (400 and 800 mg/kg) did not induce a significant difference in TAG, HDL-c, LDL-c and VLDL-c lipid profiles. However, there was significant reduction in Total cholesterol (TC) ($p<0.05$ and $p<0.001$) when treated with 400 and 800 mg/kg of *Julie* and 800 mg/kg of *Opioro* extracts. The effects of sub-acute administration of *Opioro* and *Julie* extracts of mango seed kernels on liver parameters are shown in Table 3.

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Table 3

Effects of sub-acute administration of *Opioro* and *Julie* extracts of *M. indica* L. seed kernel on lipid profiles in male Swiss albino mice.

Paramaters	Control	*Opioro*		*Julie*	
		400 mg/kg	**800 mg/kg**	**400 mg/kg**	**800 mg/kg**
Total cholesterol	103.49±2.94	100.17±2.07	98.50±2.65*	98.42±2.01*	97.34±2.87***
TAG (mg/dL)	111.80±3.38	110.93±3.10	112.11±3.32	112.16±2.90	109.95±2.42
HDL-c(mg/dL)	55.25±1.55	54.14±1.54	54.66±1.79	55.18±0.95	53.79±0.91
LDL-c(mg/dL)	25.88±3.69	23.84±3.38	21.42±2.65	21.61±1.76	21.56±2.42
VLDL-c(mg/dL)	22.36±0.66	22.18±0.62	22.42±067	22.43±0.58	21.99±0.48

Data were expressed as mean± SD, n = 5. Statistical analysis was performed using two-way ANOVA followed by Bonferreni post hoc test.

* (P<0.05) compares with respective positive controls. **** (P<0.0001) compares with respective positive controls was done with two-way ANOVA followed by Bonferreni post hoc test.

TAG, triglycerides; HDL-c, high density lipoprotein-cholesterol; LDL-c, low density lipoprotein-cholesterol; VLDL-c, very low-density lipoprotein-cholesterol.

3.6. Effects of the extracts on antioxidant enzymes

The GPx activity was significantly reduced (p<0.001) when the mice were treated with 800 mg/kg dose of *Opioro* extract compared to the control. The activities of GSH, SOD, catalase and MDA did not reveal significant changes (p>0.05) in all the treated groups (Table 4).

Table 4

Effects of sub-acute administration of *Opioro* and *Julie* extracts of *M. indica* L. seed kernels on antioxidant activity in male Swiss albino mice.

Parameters	Control	*Opioro*		*Julie*	
		400 mg/kg	**800 mg/kg**	**400 mg/kg**	**800 mg/kg**
GPx (µg/mg protein)	70.00±2.23	68.35±2.33	64.10±1.20**	72.10±4.34	66.89±2.27
GSH (µg/mg protein)	61.02±3.07	63.61±2.68	59.12±2.83	61.57±1.29	61.05±2.34
SOD (µg/mg protein)	49.33±1.42	50.22±0.76	49.47±0.63	48.50±3.51	47.97±2.10
Catalase (µg/mg protein)	32.20±1.89	32.77±1.54	30.85±0.70	31.85±1.75	32.77±1.54
MDA (µg/mg protein)	0.28±0.04	0.33±0.05	0.36±0.04	0.32±0.05	0.36±0.04

Data were expressed as mean± SD, n = 10. Statistical analysis was performed using two-way ANOVA followed by Bonferreni post hoc test.

** (p<0.001) compare with respective positive controls. **** (p<0.0001) compare with respective positive controls GPx, glutathione peroxidase; SOD, superoxide dismutase; GSH, glutathione; MDA, malondialdehyde.

Table 5

Effects of sub-acute administration of *Opioro* and *Julie* extracts of *M. indica* L. seed kernel on kidney indices in male Swiss albino mice.

Parameters	Control	*Opioro*		*Julie*	
		400 mg/kg	**800 mg/kg**	**400 mg/kg**	**800 mg/kg**
Urea (mg/dL)	9.85±0.35	9.45±0.58	11.67±1.09****	10.32±0.51	11.37±0.71***
Creatinine (mg/dL)	0.33±0.04	0.35±0.03	0.43±0.05	0.36±0.04	0.41±0.03

Data were expressed as mean± SD, n = 5. Statistical analysis was performed using two-way ANOVA followed by Bonferreni post hoc test.

*** (p<0.001) compares with respective positive controls. **** (p<0.0001) compares with respective positive controls.

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3.7. Effects of the extracts on kidney function

Opioro and *Julie* extracts produced significant increase ($p<0.001$ and $p<0.0001$) in urea activity at 800 mg/kg. compared to the control when treated with *Opioro* and *Julie* extracts at 800 mg/kg. No significant changes were observed for creatinine when the mice were treated with both extracts at 400 and 800 mg/kg doses (Table 5).

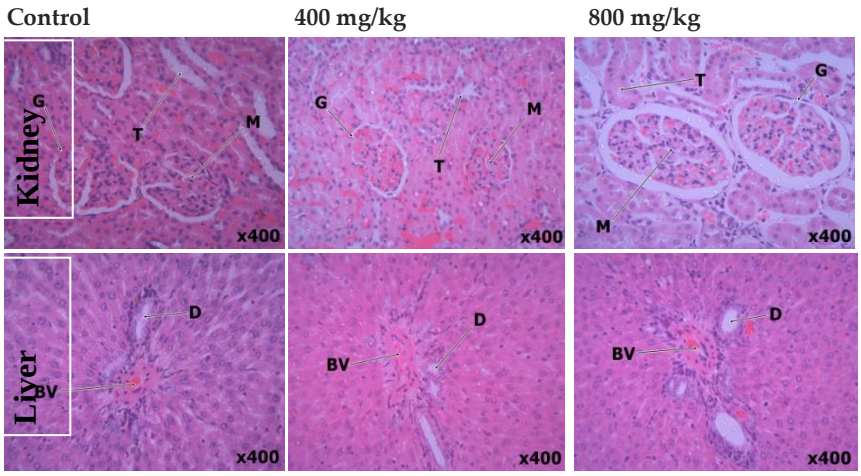

Fig. 1a. Histopathology of kidney and liver following treatment with *Julie* extract of *Mangifera indica* L. seed kernel daily for 28 days. G, glomerulus; T, tubule; M, mesangium; BV, blood vessels; D, ductile. Staining was done using H&E. No significant alteration was observed in all treatment groups (x 400 magnification).

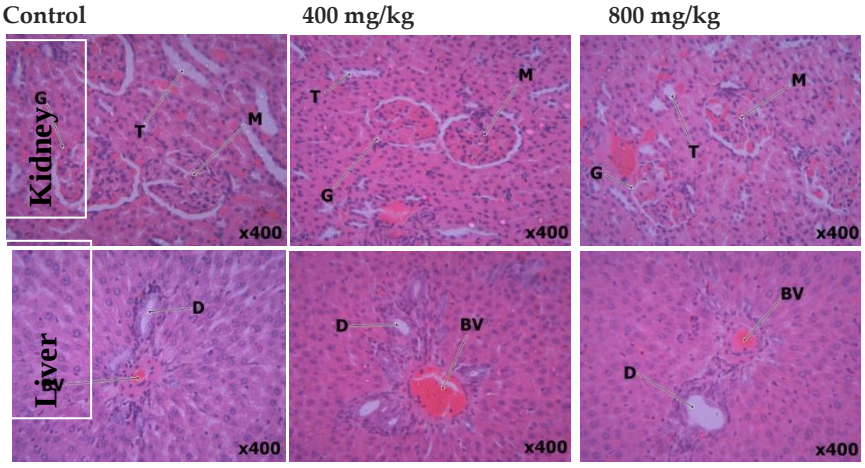

Fig. 1b. Histopathology of kidney and liver following treatment with *Opioro* extract of *Mangifera indica* L. seed kernel daily for 28 days. G, glomerulus; T, tubule; M, mesangium; BV, blood vessels; D, ductile. Staining was done using H&E. No significant alteration was observed in all treatment groups (x 400 magnification).

3.9. *Effects of the extracts on* organ histology

After 28 days of treatment, the histological examination of the organs (Figures 1a and 1b) showed no observable alterations in the glomerulus (G), tubule (T) and mesangium (M) of the kidney. The same result was observed in blood vessels (BV), and ductile (D) of the liver.

4. DISCUSSION

In spite of extensive use of herbal drugs in the management of diseases in developing countries, only few research has been conducted on safety of the therapeutic use of plant extracts (Saad et al., 2006). This study evaluated the toxicity in *Opioro* and *Julie*

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extracts in relation with biochemical and hematological biomarkers in mice as well as histopathology of vital organs. Blood is one of the most frequent targets for harmful compounds and important indicator of functional and pathological conditions (Liju et al., 2013). Toxicological studies stated that changes in hematopoietic system have a higher diagnostic value for human toxicity when the results obtained from animal researchers are compared with it (Olson et al., 2000). The hematological analysis in this study does not reveal any change in Hb, HCT, RBC, WBC, MCV, MCH, MCHC when compared to the control; however, a decrease in platelets level at 400 mg/kg when treated with *Opioro* and *Julie* extracts and 800 mg/kg for *Opioro* was observed. Platelets are responsible for blood clotting. They have a maximum life span of 9 days, and their formation is well-designed and clearly induced. In this study, significant reduction in platelet counts was revealed in the mice at higher and lower doses of both extracts. These slight alterations however, might partially impair the platelet functions (Twomey et al., 2018) but may not produce detrimental effect to the mice as the other parameters were unaffected.

The liver is an essential organ responsible for the metabolism and biotransformation of chemical substances (Muriel et al., 2017). Daily, it is exposed to internal and external agents that interfere with its normal activities leading to liver diseases (Lee, 2003). The damage of the liver, kidney and muscle cells causes the release of serum biomarker enzymes namely: AST, ALT and ALP. In this study, evaluation of these biomarkers helped determined the level of activity of the extracts on the liver. AST and ALT significantly increased at the treated doses of *Opioro* and *Julie* extracts, while there was a significant reduction in ALP at 400 mg/kg for *Opioro* extract in comparison with the control. Usually, AST and ALT work with other liver parameters such as total protein, albumin, bilirubin and globulin; these parameters from the result did not produce any significant difference, therefore, changes in AST and ALT in this study may not be adverse. However, persons with liver dysfunction should not consume the seed kernels as this may heighten the problems. The reduced value of ALP may not be clinically relevant since the change occurred at a single dose (400 mg/kg) of *Opioro* extract. This suggests that the extracts may not produce any significant adverse effect on the liver and kidney cells.

Liver is also the site of bilirubin detoxification as well as protein synthesis, which include albumin and globulin (Lee, 2003). The levels of protein synthesis in the liver are disrupted by the presence of extraneous agents. The bilirubin is the byproduct of degradation of hemoglobin in the red blood cell. Its elevated height is an indication of liver inflammation and dysfunction (Thapa and Walia, 2007). In this study, the values of protein, bilirubin, albumin and globulin did not produce any significant difference in the treated groups compared to the control, which suggest also that the two extracts did not produce adverse effect on the hepatic cells in the synthesis of protein and removal of waste.

Lipids generally are essential for the development and maintenance of the cell membrane; serve as precursors for vitamin D, bile salts and steroid hormone formation. Total cholesterol (TC) is associated with elevated atherosclerosis in the blood vessels while LDL-c and VLDL-c serve as carrier molecules, delivering the TC to certain tissues and organs. For effective management and prevention of the disease, use of drugs, food supplements and nutrition have been advocated to reduce the accumulation of TC, VLDL-c and LDL-c, and increase the level of HDL-c (NCEP, 2002; Brunzel et al.,2008; Drexel, 2006). From the study, it was discovered that HDL-c, LDL-c, VLDL-c and TG did not show any significant change when compared to control, but TC was significantly lower than the control. The low value of TC obtained from the treated mice at 400 mg/kg and 800 mg/kg for *Julie* and 400 mg/kg for *Opioro* suggest that their lipids might be composed of monosaturated oleic and stearic acids. These monosaturated fats are essential in human nutrition as they reduce the risk of cardiovascular disease and effective in the reduction of triglycerides, LDL-c, total cholesterol, and glycemic index (Kittiphoom and Sutasinee, 2013). Unlike other saturated fatty acids, stearic acid does not produce increase in the lipoprotein cholesterol level and is not harmful to the body (Yu *et al*., 1995). Therefore, these varieties when consumed as food supplements might help reduce thrombogenicity and atherogenicity (Yu *et al*., 1995).

Glutathione (GSH) is a biomolecule comprising of thiol and tripeptide synthesized in the liver by the combination of glycine, cysteine and glutamate. It has a cytoprotective function; ensuring that the body is protected from neurodegenerative diseases, AIDS, aging, diabetes mellitus, cancer and heart diseases (Wu *et al*., 2018; Townsend *et al*., 2003). This hypotensive activity occurs due to its exertive effect on the nitric oxide-free radical interaction (Ceriello et al., 1991). The first and most effective detoxification enzyme known as superoxide dismutase (SOD) is a metalloenzyme which converts harmful superoxide anion to hydrogen peroxide and molecular oxygen (Dringen et al., 2005; Fridovich, 1995). Constant consumption of SOD supplement preserves host immunity and retards aging process (Ighodaro and Akinloye, 2017). Moreover, catalase, a common antioxidant enzyme, completes the detoxification process initiated by SOD. It utilizes either iron or manganese to reduce hydrogen peroxide to water and oxygen; its shortfall predisposes the body to type 2 diabetes (Goth *et al*., 2004). On the other hand, GPx is an essential intracellular enzyme that inhibits lipid peroxidation and damage to membrane fatty acid by converting lipid and hydrogen peroxides to corresponding alcohol and water respectively (Gill and Tuteja, 2010; Markund, 1984). It stimulates the production of collagen by liver cells and protects the body from cancer and cardiovascular diseases (Ighodaro and Akinloye, 2017; Hadizadeh *et a*l., 2017). GPx, from the study was significantly lower than the control. A significant decrease in the value of GPx was observed in the mice when treated

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with *Opioro* extract at 800 mg/kg. This change, however, could not be considered toxicologically relevant since no effect was produced by *Julie* at tested doses, and no histopathological alterations were observed in the liver. From the study, the levels of GSH, MDA, SOD and catalase did not vary with those of control. Thus, indicating that the extracts at the treated doses did not produce any rise or decrease in the levels of the antioxidant enzymes in comparison with the control; thus, suggesting that both *Opioro* and *Julie* may have no adverse effects on the liver cells as there were no significant fall in their level.

Renal failure is a condition that leads to gradual and steady decrease in the performance of kidney due to some genetic and other degenerating diseases like cancer, diabetes and hormonal disorders (Eduardo *et al*., 2015). The progression of kidney damage is ascertained by the rise in two serum biomarkers, urea and creatinine whose presence in serum helps to determine glomerular filtrate and renal function. Urea is a nitrogenous waste produced from the breakdown of proteins in diets by the liver and circulated in the blood (Corbett, 2008). Similarly, creatinine is the outcome of creatine phosphate degradation in muscle whose concentration is dependent on the muscle mass. Increased serum urea and creatinine levels are associated with kidney disease, blockage of urinary tract, congestive heart failure (Zou *et al*., 2008) and ureamia (Entedhar and Nawal, 2016). In this study, the urea activity significantly increased at 800 mg/kg doses of both *Opioro* and *Julie* extracts. Therefore, dose higher than 800 mg/kg may pose potential risk. The significant values of urea obtained are positive indication of the high protein content (Mitchell and Kline, 2006) in these extracts; however, this is very detrimental as repeated consumption can lead to accumulation of urea, which develops, into heart malfunction, kidney and gall stones and hemorrhage in the gastrointestinal tract (Gwoda *et al*., 2010). There were no significant differences in creatinine level obtained in the study, in comparison with the control group. This is an indication that the extracts at the tested doses cannot trigger hyperthyroidism, blood shortage, leukemia and stroke [63].

In this study, the toxicological effect of *Julie* and *Opioro* extracts on the histology of vital organs in male Swiss albino mice was examined. The result obtained showed no alteration in the glomerulus (G), tubule (T), mesangium (M) of the kidney. The same was also observed in blood vessel (BV), ductile (D) of the liver even at 400 mg/kg and 800 mg/kg of the two extracts. This finding suggests that seed kernel extracts of *Opioro* and *Julie* have no toxic effect on the most vital organs of mice (kidney and liver), thus, they could be considered safe for consumption when further clinical tests are performed on human model.

5. CONCLUSION

This study demonstrated that *Opioro* and *Julie* seed kernel extracts did not produce severe changes in mice at normal doses, evidently seen in the histopathological study. However, further clinical investigations are needed to ascertain their safety and effectiveness in humans.

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Authors' Contribution

The conceptualization of the paper as well as the methodology of the work was created by Ibe, Chibuike. The manuscript was written and the experiment performed by Nwachukwu, Kingsley C. The collection of data and statistical analysis were performed by Nwachukwu, Kingsley C. Nwachukwu, Nkechi C., supervised and proofread the first draft and Ugbogu, Ositadinma C. proofread and edited the final draft.

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Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Anand U, Jacobo-Herrera N, Altemimi A, Lakhssassi N. A Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug Discovery. *Metabolites*. 2019;9(11):258
2. Radha MK., Puri S, Pundir A, Bangar SP, Changan S, Choudhary P, Parameswari E, Alhariri A, Samota , M. K., Rahul D. Damale, R. D., Singh, S., Berwal, M. K., Dhumal, S., Bhoite, A. G., Senapathy, et al. 2021. Evaluation of Nutritional, Phytochemical, and Mineral Composition of Selected Medicinal Plants for Therapeutic Uses from Cold Desert of Western Himalaya. *Plants* 2021;10:1429.
3. Newman DJ, Cragg GM. 2020. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *J Nat Product*. 2012;83(3):770-803.
4. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. *Molecules*. 2016;21:559.
5. Torres-Leon C, Rojas R, Contreras-Esquivel JC, Serna-Cock L, Belmares-Cerda R, Aguilar CN. Mango seed: functional and nutritional properties. *Trends Food Sci and Technol*.2016;55:109-117.
6. Arogba SS, Omede A. 2012. Comparative Antioxidant Activity of Processed Mango (Mangifera indica) and Bush Mango (Irvingia gabonensis) Kernels. *Niger Food J*. 2012;30(2):17– 21.
7. Mwaurah P, Kumar S, Kumar N, et al. Physicochemical characteristics, bioactive compounds and industrial applications of mango kernel and its products: A review. *Compr Rev Food Sci Food Saf*. 2020;19(2):1-26.
8. Awad El-Gied AA, Joseph MRP, Mahmoud IM, Abdelkareem AM, Al Hakami A, Hamid M. Antimicrobial Activities of Seed Extracts of Mango (Mangifera indica L.). *Adv Microbiol*. 2012;2:571-576.
9. Lebaka VR, Wee Y, Ye W, Korivi M. Nutritional Composition and Bioactive Compounds in Three Different Parts of Mango Fruit. *Int J Environ Res Public Health* 2021;18:741.
10. Abdel-Aty AM, Salama WH, Hamed MB, Fahmy AS, Mohamed SA. Phenolic-antioxidant capacity of mango seed kernels: Therapeutic effect against viper venoms. *Rev Bras Farmacogn*. 2018;28:594-601.
11. Mutua KM, Imathiu S, Owino W. Evaluation of the Proximate Composition, Antioxidant Potential, and Antimicrobial Activity of Mango Seed Kernel Extracts. *Food Sci & Nutr*. 2016;5(2):1-9.
12. Anyanwu MU, Okoye, RC. Antimicrobial activity of Nigerian medicinal plants. Journal of *Intercultural Ethnophamacol*. 2017;6(2): 240-259.
13. Aguoru CU, Ajah P, Olosan JO. Taxonomical Investigation of four varieties of *Mangifera* Using Micro-Anatomic Features. *Int Res J Nat Sci*. 2016;4(1):20-27.
14. National Research Council Committee for the update of the Use of Animals. The National Academics Collection: Reports Funded by National Institutes of Health, Guide for the Care and Use of Laboratory Animals. The National Academic Press (US). 2011.
15. Organization for Economic Co-operation and Development (OECD). Guidelines for Testing of Chemical, Guideline 423. Acute Oral Toxicity-Acute Toxic Class Method (Paris). 2001
16. Organization for Economic Co-operation and Development (OECD). Test No. 407: repeated dose 28-day oral toxicity study in rodents. 2008
17. Akomas SC, Okafor AI, Ijioma, SN. Hypoglycemic, Hematologic and Hypolipidemic Activity of *Mucuna pruriens* ethanol leaf extract in alloxan induced diabetic rats. *Annu Res Rev Biol*. 2014;4(24):4284–4292.
18. Aebi H. Catalase in vitro. *Meth Enzymol*. 1984;105:121–126.
19. Górny M, Bilska-Wilkosz A, Iciek M. et al. Alterations in the Antioxidant Enzyme Activities in the Neurodevelopmental Rat Model of Schizophrenia Induced by Glutathione Deficiency during Early Postnatal Life Magdalena. *Antioxidants*. 2020;9:538.
20. Ohkawa H, Ohshi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*.1979; 95:351–358.
21. Saad B, Azaizeh H, Abu-Hijleh G, et al. Safety of traditional Arab herbal medicine. *Evidence-based Complement Altern Med*. 2006;3433–439.
22. Liju VB, Jeena K, Kuttan R. Acute and subchronic toxicity as well as mutagenic evaluation of essential oil from turmeric (*Curcuma longa* L). *Food Chem Toxicol*. 2013;53–52–61.
23. Olson H, Betton G, Robinson D, et al. Concordance, of the toxicity of pharmaceuticals in humans and in animals. *Regul Toxicol Pharm*. 2000;32(1):56–67.
24. Twomey L, Wallace RG, Cummins PM, et al. Platelets: From Formation to Function, *Homeostasis* 2018;1–19.
25. Muriel P. Liver Pathophysiology: Therapies and Antioxidants. Academic Press, Mexico City, Mexico. 2017: 24–26.
26. Lee W M. Acute liver failure in the United States. *Semin Liver Dis*. 2003;23(3):217-226.
27. Thapa BR, Walia A. Liver function tests and their interpretation. *Indian J Pediatr*. 2007;74: 663–671.
28. NCEP (National Cholesterol Education Program). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

Treatment of High Blood Cholesterol in Adults–Adult Treatment Panel III, Final Report, NCEP,National Heart, Lung, and Blood Institute, National Institutes of Health, USA. 2002.

29. Brunzell JD, Davidson M, Furberg CD, et al. Lipoprotein management in patients with cardiometabolic risk–consensus statement from the American Diabetes Association and the American College of Cardiology Foundation. *Diabetes Care*. 2008;31:811–822.
30. Drexel H. Reducing risk by raising HDL-cholesterol: the evidence. *Eur Heart J Suppliment*. 2006;8:23–29.
31. Kittiphoom S, Sutasinee S. Mango seed kernel oil and its physicochemical properties. *Int Food Res. J*. 2013;20(3):1145–1149.
32. Yu S, Derr J, Etherton TD, et al. Plasma Cholesterol-Predictive Equations Demonstrate That Stearic Acid Is Neutral and Monounsaturated Fatty Acids Are Hypocholesterolemic. Am J Clin Nutr. 1995;61:1129–1139.
33. Wu T, Gao Y, Guo X, et al. Blackberry and Blueberry Anthocyanin Supplementation Counteract High-Fat-Diet-Induced Obesity by Alleviating Oxidative Stress and Inflammation and Accelerating Energy Expenditure. *Oxid Med Cell Longev*. 2018;4051232.
34. Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. *Biomed. Pharmacother*. 2003;57:145–155.
35. Ceriello A, Giugliano D, Quatraro A, et al. Anti-oxidants show an anti-hypertensive effect in diabetic and hypertensive subjects. *Clin Sci*. 1991;81:739–742.
36. Dringen, R, Pawlowski PG, Hirrlinger J. Peroxide detoxification by brain cells. *J Neurosci Res*. 2005;79:157–165.
37. Fridovich I. Superoxide radical and superoxide dismutases. *Annu Rev Biochem*. 1995;64:97–112.
38. Ighodaro OM., Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria J Med*. 2017;54(4):1–7. Goth L, Rass P, Pay A. Catalase enzyme mutations and their association with diseases. Molecular diagnosis. 2004;8:141–149.
39. Gill SS, Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem*. 2010;48:909–930.
40. Marklund SL. Extracellular superoxide dismutase and other superoxide dismutase isoenzymes in tissues from nine mammalian species. *Biochem. J*. 1984;222:649–655.
41. Hadizadeh F, Faghihimani E, Adibi P. (2017). Nonalcoholic fatty liver disease: Diagnostic biomarkers. World J Gastrointest Pathophysiol. 2017;8–11.
42. Eduardo OC, Kaue A, Idania AA. Influence of hemodialysis on the plasma concentration of adenosine deaminase in patients with chronic kidney disease. *J Bras Patol Med Lab*. 2015;51:153–157
43. Corbett JV. Laboratory tests and diagnostic procedures with nursing diagnoses. (7th Ed.). Pearson, University of San Francisco. 2008:90–107.
44. Zou Y, Wang C, Zhou J, et al. Simultaneous Determination of Creatinine and Uric Acid in Human Urine by High Performance Liquid Chromatography. *Anal Sci*. 2008;24:1589–1592.
45. Entedhar RS, Nawal AM. Biochemical changes in chronic renal failure pre and post hemodilysis. *J Environ Sci Eng*. 2016;5:190–195.
46. Mitchell HR, Kline W. Core curriculum in nephrology, Renal Function Testing. *Am J Kidney Dis*. 2006;47:174–183.
47. Gowda S, Desai PB, Kukarni SS, Hull VV, Math AAK, Vernekar, SN. Markers of renal function tests. *N Am J Med Sci*. 2010;2:170–173.

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS